ON THE INTERSTITIAL CELLS OF THE TESTICLE IN DIDELPHYS.

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I completed a short time ago a study of the seminal cells of *Didelphys*, to appear (1919) in Contributions to Embryology, published by the Carnegie Institution of Washington, in a memorial volume dedicated to the late Professor Franklin P. Mall. While making that investigation I was struck by the large number of interstitial cells present in the testicle of the opossum, and, upon closer observation, by the complexity of their structure. I therefore determined to undertake a study of these cells.

The material upon which it was based consisted of nine animals. Five were full grown, a sixth exhibited all stages of spermiogenesis except the very last—*i. e.*, the formation of the spiral filament at the expense of the chondriosomes—and the remaining three were in a much less advanced stage of development. Fragments of the testicle were fixed in the following fluids: Hermann's, Ramon y Cajal's mixture of formalin and uranium nitrate (1912), Bouin's, Flemming's, Altmann's, Meves', Benda's, Regaud's, acetic sublimate and saturated sublimate. Two adult animals were sacrificed in order to study the living cells, and were injected with a solution of janus-green (1–10,000 in 0.85 per cent. salt solution). I am greatly indebted to Professor E. V. Cowdry for his help in carrying out these experiments.

Of the above mentioned reagents Benda's, Meves', Regaud's and Altmann's fluids were used for the purpose of fixing the chondriosomes. To stain these bodies iron-hematoxylin, Benda's and Altmann's methods, and a combination of acid fuchsin and methylgreen were employed. Ramon y Cajal's mixture of formalin and uranium-nitrate was applied in order to bring into evidence the apparatus of Golgi. The best preparations were

obtained by leaving the pieces in the fixative for 9 hours, in the silver nitrate 37 hours, and in the developer 14 hours. Instead of other complicated procedures, at the suggestion of Professor Cowdry I used the following method in the treatment of the sections: The slides were first immersed in 0.1 per cent. gold chloride for 2–3 hours; then in 5 per cent. hyposulphite 20–30 minutes. The results were excellent. Probably any nuclear dye could be used as a counterstain. I resorted to Ehrlich's hematoxylin, safranin, and methyl green (Cowdry, 1916), which in a concentration of one half per cent., applied for 30–60 seconds, gives a very sharp contrast to the black color of the apparatus and the pale background. For reasons to be made clear later on, special methods (Mallory's, safranin-light green, and a combination of Congo red, iron-hematoxylin and light green) had to be used for staining the connective tissue. In the application of these, especially of the latter method, Professor Van der Stricht kindly gave me the benefit of his wide experience. It may be worth while to state in passing that Mallory's method gives just as good if not better results after fixation with Flemming's fluid (modifications of Benda and of Meves included) as after sublimate or similar fixatives.

Concerning the structure of the interstitial cells in the opossum, I find in the literature only two short notes; one by Whitehead (1908), the other by Jordan (1911). Whitehead states that these cells contain no fat. Jordan represents the mitotic division of the interstitial cells and mentions their chondriosomes. Data on the structure of interstitial cells in other mammals, on the other hand, are abundant and will be referred to when occasion arises.

In accord with the description of a number of authors, notably Reinke (1896), Lenhossek (1897), Bouin and Ancel (1903), and Winiwarter (1912, 1), I found both single and double-nucleated interstitial cells (Figs. 2 and 7), although the latter are of far less frequent occurrence. Most of the nuclei show a marked depression on one side (Figs. 1, 3, 5, 9 and 10); only in material fixed with Regaud's fluid is this peculiarity of structure not visible, probably because the nuclei swell somewhat in this fixative. The process of formation of the double nuclei has not

been observed but is probably amitosis.   Perhaps the nuclear depression just mentioned may be considered as an indication of direct division, but it must be stated that the number of cells in which this was noted greatly exceeded the number of binucleated cells.   Mitotic division of the interstitial cells in the opossum, although observed occasionally, is rare.   Jordan (1911, Fig. 7) has mentioned it.

The structure of the groundsubstance of the protoplasm varies considerably after the different fixatives.   After all reagents that preserve the chondriosomes it appears homogeneous and exhibits a remarkable affinity for a number of dyes.   In preparations made after Benda's method it stains intensely with sulfalizarin. Acid fuchsin is retained by it so tenaciously that the seminal epithelium usually has to be almost entirely unstained in order to obtain the proper grade of differentiation in the interstitial cells.   Mulon (1911, 1 and 2) describes in the cells of the cortical suprarenal, in the corpora lutea and in the interstitial cells of the ovary, an amorphous, siderophile or osmiophile substance which, he supposes, is formed by the coalescence of chondriosomes.   This substance he considers as a secretion which accumulates in the cell and is finally eliminated in bulk.   Athias (1911), however, believes in the presence of a diffuse lipoid formed at the expense of poorly preserved chondriosomes.   It is possible that the diffuse substance observed in my material may be the product of secretion, as we shall later see, but it certainly has nothing to do with artificial or natural disaggregation of the chondriosomes, for these bodies appear well preserved within it.

Lenhossek (1897) was the first author to describe the centers of the interstitial cell.   In man he describes a darker inner zone— an "endoplasm" which corresponds obviously to the idiosome,[1] although he was not able to demonstrate the presence of centrioles within it.   These have been recently brought into evidence in human material by Winiwarter (1912, 1), who found them to be rod-shaped.   In the interstitial cells of the cat Lenhossek undoubtedly saw both the idiosome and the centrioles.   In the opossum I find that there exists in most of the interstitial cells,

[1] In future I shall adopt the spelling *idiosome*, as proposed by Regaud (1910), instead of *idiozome*, for the reasons assigned by that author.

in close proximity to the nucleus, a differentiation of the cyto-plasm (Figs. 1, 2 and 8). This zone contains the centrioles of the resting cell and is undoubtedly to be considered as an idio-some. In contradistinction to what one observes in the seminal cells, the idiosome of the interstitial cells is not sharply delimited by a special cortical layer. It appears most clearly in material fixed with Bouin's fluid or with any reagent containing osmic acid. It is frequently found located in the nuclear depression, as Ballowitz (1898, 1900) observed it, but not always so (for in-stance, Fig. 1). While two centrioles exist in the cells provided with only one nucleus, I find, as did Winiwarter in human material, that binucleated cells have four centrioles, and in some cases these are obviously rod-shaped (Fig. 2).

The extreme results obtained for the apparatus of Golgi by the Ramon y Cajal method are illustrated in Figs. 3 and 4.[1] In the first, the apparatus appears as a dense reticulum, in the second the reticulum is much looser. Normally, the apparatus is concentrated at one pole of the nucleus; cases like the one represented in Fig. 4, in which one or two branches of the net-work extend to the opposite pole and surround the nucleus com-pletely, are exceptional. The apparatus is found either in the nuclear depression or in another place (Fig. 3). It behaves in this respect like the idiosome, and consequently nothing definite can be said concerning the topographical relationship of these two bodies. Nothing, however, would seem to militate against the existence of a close relationship such as has been established for a large number of other cells (for literature, see Duesberg, 1914, 1919). Other investigations will probably help to solve the problem, the present one being, to the best of my knowledge, the first on the apparatus of Golgi in the interstitial cells of the testicle. Concerning the interstitial cells of the ovary, we have the data of Cattaneo (1914) and Kulesch (1914).

While the interstitial cells of the opossum contain little fat, it would not be correct to say, with Whitehead, that they contain none at all. As a matter of fact, while the number of fat drop-lets is usually small, it varies with each animal. In other mam-

[2] Another drawing of an interstitial cell in the testicle of the opossum, with the apparatus of Golgi, will appear in another paper (1919, Fig. 34).

mals (rat, guinea-pig, rabbit, dog and man) Ciaccio (1910) describes a number of granules and vesicles which, after fixation with his special methods, stain with Sudan. Ciaccio considers these bodies as formed by a lipoid substance which, in his opinion, has a great analogy to the substance of the chondriosomes. Although none of my material was fixed exactly according to Ciaccio's prescription, I applied his method of staining to material fixed in Regaud's fluid and subsequently kept in 3 per cent. bichromate for a week, a procedure which is similar to Ciaccio's method 1. The results were identical with those obtained from preparations fixed with reagents containing osmic acid; that is to say, Sudan apparently stained in red what would be stained black in osmic acid. This conclusion was reached through the study of the seminal epithelium, in which the fat droplets have a characteristic arrangement, rather than through the study of the interstitial cells in which no such regular arrangement exists.

Pigment, which has often been found in other mammals and quite lately in the woodchuck by Rasmussen (1917), does not appear to exist at all in the interstitial cells of the opossum. Crystalloids, on the other hand, are found in many cells and are of two types. So far, those described for the first time by Reinke (1896) are supposed to exist only in human material, although Mathieu (1898) mentions the presence of "filaments cristalloïdiens" in the interstitial cells of the testicle in the cat. The plates accompanying his paper, however, are by no means clear. In the opossum many interstitial cells contain bodies similar to Reinke's crystalloids, although smaller and not so abundant (Fig. 6). They appear most clearly after fixation with Benda's or Meves' fluid, and can be seen in all other material except that fixed with Regaud's reagent; after the application of Benda's method they usually stain in brown. These bodies are rather short and thick; their ends are not pointed but blunt. Frequently they appear to be formed of two substances—a darker peripheric, and a lighter central one. In most cases only one crystalloid is found in a cell; occasionally, however, their number is increased, although they are never so abundant as Reinke's crystalloids in man. Sometimes a large crystalloid

will appear to be split at one end. The resemblance of these bodies to the crystalloids of Reinke is accentuated by the fact that, in rare cases, they are found surrounded by a clear space, located in a sort of vacuole.

The second type is not quite so frequently noted as that just described. It is represented by what appear to be thin rods, pointed at both extremities, which vary considerably in length in the different cells. The shorter ones are usually straight, the larger ones curved. In most of the cells they are multiple (Fig. 5). These, too, are seen most clearly after fixation with Benda's fluid, but are preserved in other fixatives also. In Regaud's preparations, however, I was never able to find them. Similar bodies exist in the cells of Sertoli. If I were to venture a comparison it would be to liken them to the bodies described by Lubarsch in the human testicle under the name of *Charcot-Böttcher's crystals.* The so-called Lubarsch crystals are, as Lenhossek has pointed out, located in the spermatogonia, but there is in the seminal epithelium another form of crystalloids, also described by Lubarsch (the Charcot-Böttcher crystals), which are larger and are located in the cells of Sertoli. According to Montgomery (1911) and to Winiwarter (1912, 2), the two forms are in genetic relation. Montgomery, who unfortunately failed to compare his observations with those of previous authors, described them in the "antepenultimate spermatogonia" of man as "rods," and came to the interesting conclusion, corroborated by Winiwarter (1912, 2), that "the presence of the rod determines the line of the Sertoli cell" (p. 368). Crystalloids have also been found in the Sertoli cells of the pig by Bouin and Ancel (1903, Fig. 11) and possibly in the cat by Hague (1914). I did not see anything resembling the numerous small crystalloids described by Lenhossek (p. 68, Figs. 2, 3 and 4), which correspond, in my opinion, to Winiwarter's (1912, 1) "grains riziformes."

The chondriosomes of the interstitial cells in mammals have been seen by Jordan (1911), Winiwarter (1912, 1), and Rasmussen (1917). As stated above, Jordan merely mentions their presence in the opossum. Winiwarter gives a longer account of these bodies in man, where he finds both granules and rods. Rasmussen describes in the woodchuck small granules, "the only

thing of a mitochondrial character that could be demonstrated in the interstitial cell at any time," a description which is rather unsatisfactory.   My own observations show that the chondrio-somes are exceedingly numerous and that they are mostly rods, short and curved.   They are crowded into several heaps, some of which are often seen in close proximity to the nucleus.   Fig. 7, drawn from a preparation fixed and stained after Benda, gives what should be considered their natural aspect, as they appear exactly alike in teased preparations after injection of janus-green. It is surprising how easily the interstitial cells take up the vital dye, in contradistinction to the seminal cells, which it is very difficult to stain in vivo: the reason for this difference is ob-viously the presence of a sheath of connective tissue around the seminiferous tubule.   After fixation in Regaud's fluid the ap-pearance is not altered; it must be stated, however, that after the action of that fluid, which has a great power of penetration, the chondriosomes can be well preserved all through the piece, while in Benda's material they are well fixed only in a very thin layer of tissue, at the periphery.   The chondriosomes seem to be especially labile in the interstitial cells.   As usual, the figures of deformation are, in the first stages, granules and vesicles.   Of other forms, which I am led to consider as related to the chondri-osomes, I shall speak later.

Winiwarter (1912, 1) mentions the existence of transitional forms between the chondriosomes and the crystals, but does not conclude definitely in favor of a genetic relationship.   As an argument in favor of such a relationship one could bring into the field the following observations of that author: In the fetus chondriosomes only are present in the interstitial cells, the other elements appearing afterward.   I regret to say that I have no information to offer on this point.

In all my material, except when again fixed with Regaud's fluid, cells are found, which contain a number of granules. These granules can be stained by different methods.   I am strongly inclined to consider them as a secretion product, a question which will be taken up again shortly.   As stated, these granules are not constant nor are they very abundant in one cell: not to be compared with the bulky masses represented by Bouin and Ancel (1903) for instance, in Fig. 7 of their paper.

We come now to the description of a most interesting condition
in these interstitial cells of the opossum, a condition which I
noted first in preparations stained and fixed after Benda, where
it is most conspicuous. It can be seen also, however, in other
preparations, such as Flemming's, Meves', Hermann's and
Bouin's. A substance, which sometimes appears granular, at
other times amorphous, fills the intercellular spaces. Its quan-
tity is variable; some interspaces show so little of it as to escape
notice, others are widely dilated by the presence of large quanti-
ties of the substance, as shown in Fig. 13. It is most conspicuous
in preparations made after Benda's method because it takes up
avidly the crystal-violet, in striking contrast to the light-brown
color of the background and of the nuclei. The same substance
stains intensely with safranin and iron-hematoxylin; in the
Mallory method it takes up the orange and is consequently
rather inconspicuous.

A closer investigation shows that from these intercellular
spaces processes penetrate into the cell-body (Figs. 12 and 13).
While most of these processes look like the substance accumu-
lated in the intercellular spaces, some have the sharp appearance
of a more definite structure, such as laminæ of some sort, and
my first supposition was that I had to do with processes of con-
nective tissue penetrating into the cell-body, such as have been
described for large nerve-cells. A study of preparations stained
especially for the connective tissue failed, however, to substan-
tiate this opinion. After Mallory's stain, for instance, I could
not find any such process electively stained in blue. While the
thinnest fibrils of connective tissue in the sheath of the vessels
and the connective membrane of the seminiferous tubules gave
a typical collagenous reaction, in the same preparations the
intracellular processes always were stained in orange like the
intercellular substance. As a matter of fact, the study of these
preparations corroborates Lenhossek's description (p. 78). The
interstitial cells lie in heaps or irregular rows separated by the
larger vessels. It is quite exceptional to see a process of the
connective sheath of these vessels penetrate into a group of
interstitial cells. The cells are, as a rule, separated only by
intercellular spaces, virtual or real, and perhaps also in certain

places by small capillaries.   I conclude, therefore, that the intra-
cellular processes are formed by the substance filling the inter-
cellular spaces, a substance which, in my opinion, represents
the product of secretion.   If this interpretation be correct, as
I believe it to be, here is a remarkable instance of a gland with
internal secretion, in which the product of secretion can be
followed, owing to its staining qualities, from within the cell
into the passage of excretion.   Favorable sections, like that
represented in Fig. 13, carry one even a step farther and show the
same product in a vessel.

What, then, is the real nature of these intracellular and inter-
cellular spaces, and of these vessels with which the intercellular
spaces seem to be in some way connected?   The penetration of
lymphatics into a cell, not to speak of the much discussed and
very disputable conditions described by Adamkiewicz (1886,
1900), Browicz (1902, 1 and 2), Schäfer (1902, 1903), Schlater
(1902) and others, has been repeatedly reported.   At first Holm-
gren interpreted his trophospongium as such.   Among the more
recent observations I may mention those published by Ciaccio
(1903), Felicine (1904), Kumita (1909) and Matsunaga (1909).
The first three authors studied the suprarenal.   Ciaccio, after
the application of Golgi's method, and Felicine and Kumita,
as a result of injections, come to the conclusion that there exists
a pericellular net of capillaries which send processes into the cells.
Matsunaga has published similar results after his study of the
thyroid by the injection method.   Kumita, however, wonders
whether these intracellular processes are really vessels, or "ob
sie nicht durch einen sekretorischen Zellzerfall zustande gekom-
men sind" (p. 325).   This last hypothesis, however, in his
opinion can hardly be reconciled with the fact that the injected
mass never spreads over all the cell, and that the intracellular
space has a definite shape, ending often in a swelling in the neigh-
borhood of the nucleus.   No matter what the interpretation
may be in these cases, it appears to me that the intracellular
processes in the interstitial cells of the opossum are not well
defined canals of any kind—neither processes of lymphatics nor
intracellular ducts.   They are always exceedingly irregular in
appearance and are never seen in an empty condition, but always

as processes of the intercellular substance. No structure, at least no visible structure, appears to be the substratum of these processes. They represent, in my opinion, something like the "sekretorischer Zerfall" supposed by Kumita, or the accumulation along certain lines, perhaps in connection with protoplasmic currents, of the product of secretion. Nor do the intercellular spaces represent capillaries. Their irregular shape and the fact that no endothelium seems to line them, show that they are, as I have called them so far, merely intercellular spaces which become more or less dilated according to the quantity of secretion which is accumulated in them. Finally, that the space into which this substance is poured is a capillary cannot be doubted, for it is lined with a well-recognizable endothelium. Whether, on the other hand, this capillary is a lymphatic or a bloodvessel is not so easy to tell, and must be decided by methods other than those employed by me. Nor do the bibliographic data give any definite clue. Regaud (1897), who has made a special study of the distribution of the lymphatics in the mammalian testicle, finds that it is exceedingly variable in the different species. He distinguishes three types: One in which the lymphatic net is exclusively peritesticular; another in which the lymphatics penetrate as far as the corpus Highmori and the interlobular septa; and a third, in which the lymphatics form a network around the seminiferous tubules. It may be worth while to determine sometime, in connection with the problem of the excretion from the interstitial cells, to which type the opossum belongs.

To sum up, the product of secretion is accumulated in the interstitial cell, then discharged into the intercellular spaces, and from there passes into the circulation. Although this is one of the clearest instances in which the secretion product of a gland with internal secretion actually could be followed from the glandular cell into the vascular system, owing probably to the favorable staining properties of the secretion product, there are similar observations in the literature. Reinke has already called attention to the common staining properties of his crystals and the testicular lymph, and concludes in favor of the passage of the substance of these crystals into the lymphatics. Lenhossek

mentions (p. 78) the presence of a coagulum into the "lymphatic spaces" of the human testicle, and Sénat (1900, p. 65) writes: "Chez tous les rats dont les testicules ont été examinés, environ une dizaine, C. Regaud a rencontré dans les espaces conjonctifs une substance particulière, évidemment coagulée par les réactifs. . . . Cette substance a les mêmes réactions colorantes que le contenu protoplasmique des cellules interstitielles.   Elle forme des flaques, parfois très étendues, qui englobent ces cellules. Cette substance parait être le produit de secrétion des cellules interstitielles; peut-être résulte-t-elle de leur désintégration." Bouin and Ancel (1903) state: "Une fois gorgées de leur produit de secrétion, les cellules l'expulsent au dehors; aussi le retrouve-t-on en grande abondance dans les espaces laissés libres entre ces cellules. . . . De plus, on voit également de semblables formations dans les vaisseaux sanguins et lymphatiques" (p. 476).

Something similar was found by the same authors (1904) in their " cellules à granulations xanthiques;" that is to say, special interstitial cells which appear in the testicle of the horse fetus. Here, however, the process goes so far that after the excretion, "la cellule est réduite à son noyau et à une faible quantité de cytoplasme, jaune noirâtre après emploi de la méthode de Benda. Nous n'avons pu discerner si ces éléments étaient susceptibles de recommencer un nouveau cycle secrétoire ou s'ils disparaissent après la phase d'excrétion, à la manière des cellules glandulaires holocrines" (pages CXLIV–CXLV).   Finally, Popoff (1909), describing the interstitial cells in a human fetus 14 cm. long, says (pp. 447–448): "Dans un grand nombre de cellules, on observe des formations vacuolaires, à contours précis, entourant partiellement le noyau ou occupant au sein du protoplasme une place quelconque.   Le nombre, la forme et les dimensions de ces vacuoles sont des plus variables.   Tantôt, elles se présentent sous formes de fentes étroites et allongées, à direction incon- stante;   tantôt c'est un petit canal irrégulièrement calibré, incurvé ou une lacune en forme de croissant diversement orientée.   Des formations analogues, peut-être en communica- tion avec les précédentes, se rencontrent encore entre les cellules. Le tissu interstitiel est traversé par un grand nombre de capil- laires sanguins, dont quelques-uns ont à peine le diamètre d'un

globule rouge. Un examen attentif révèle un rapport étroit des capillaires avec les espaces intercellulaires."

Similar observations on other glands with internal secretion have been made, notably the following: Hultgren and Andersson (1899) describe the secretion products in the medullar part of the suprarenal in mammals as granules which are expelled into the bloodvessels (see pp. 268–269): "Dies geschieht entweder durch das Wandern der kleinen Körner durch die Wandung der Gefässe, wie dies besonders an den Venen beobachtet wird, oder, es wird das Endothel der Capillaren an gewissen Stellen zersprengt und die Zellen treten mit dem Gefässlumen in direkter Verbindung."

In this case the fate of the secretion product is very clearly shown. Colson (1910) finds in the same gland intercellular spaces filled with the substance secreted by the cells. Apparently the cell-bodies are, to a certain extent, liquified, since there appear to be no definite boundaries between cells and intercellular spaces. These spaces are, in Colson's opinion, the same that Kumita, as reported above, found in communication with the lymphatic system. Van der Stricht (1912), in the corpus luteum, finds also that the secretion product of the lutein cells is accumulated in the intercellular spaces; in some cases this intercellular space is an axial cavity around which the cells are oriented as in an epithelium.

Finally, there are within the interstitial cells certain very peculiar constituents, which are represented in Figs. 6, 9, 10, 11 and 13. They have the appearance of a sort of huge network. In a number of cells the meshes of this network are small and the trabeculæ are thin, while in others the meshes are wide and the trabeculæ quite thick. The extremes are shown in Figs. 9 and 11, but all stages of transition between these two types can be found; one of these is seen in Fig. 10. The resemblance of this network to some apparatuses of Golgi is indeed very striking, but the results obtained with the method of Ramon y Cajal, which have already been described, show that the apparatus of Golgi in these cells is something entirely different. There is also a certain resemblance to some of the trophospongia pictured by Holmgren (1904), a point which will

shortly be discussed.   In most cases, it is quite apparent that this peculiar formation does not invade the entire cell-body, but leaves two parts free; first, a space located in the immediate neighborhood of the nucleus, which it is safe, I believe, to consider as corresponding to the idiosome; second, the periphery of the cell.   There are, however, exceptions to the latter rule, as in a number of cases some trabeculæ are seen very close to the cell-boundary.

The question arises: is there any connection between this network and the intracellular accumulations of the secretion product?   In many cases it would appear as though both formations were in continuity, and that consequently the network were merely an extension of the intracellular processes throughout the cell-body.   I am, however, of a different opinion.   To me the network is the product of a transformation of the chondriosomes under special conditions of imperfect preservation.   I am perfectly aware that no such forms have been described heretofore.   The only thing with which I can, to a certain extent, compare these structures are the chondriosomes in the seminal cells of Scolopendra cingulata as represented (in my opinion imperfectly preserved) by Bouin (1905) especially in his Figs. 13, 14 and 15.   The basis for this interpretation is that this network is found only in material fixed with Benda's or Meves' fluid (after Altmann's fixation the interstitial cells kept the acid fuchsin with such tenacity that no definite conclusion could be reached), but only in the deeper parts of the pieces; that is, where the chondriosomes are not well preserved.   It is not found in material fixed in Regaud's fluid, but I assume that, as the latter reagent penetrates better than those just mentioned, it never gives the chondriosomes a chance to produce such distorted figures.   As a matter of fact, they are pretty well preserved all through the pieces, and the only modified forms that appear in that material are the vesicles—the well-known first stages of chondriolysis.   On the other hand, the secretion product is preserved by a number of reagents which do not fix the chondriosomes.   The staining reactions are also different and point toward the chondriosomal nature of the network.   Generally speaking, the network is stained in purple after Benda's method, that is,

like the product of secretion (the very large networks, such as
that represented in Fig. 11, may make exception and stain with
alizarin); but if we stain with safranin, we find that the secretion
product alone takes up the dye and stands out conspicuously.
A second reason which argues in favor of my interpretation is
the existence of all possible transition forms between the net-
work and the first stages of chondriolysis.   In the cell represented
in Fig. 8 there are some of those vesicles which, as we know,
result from the artificial fragmentation and swelling of the chon-
driosomes.   There are present also some forked forms, which
are probably produced by the confluence of the swollen chondrio-
somes, and give an indication of how a network can be built at
the expense of the normal, independent chondriosomes.   Again,
although at times the network appeared to be connected with
the intracellular accumulations of secretion, on closer examination
no such connection could be actually established.   Finally, there
is a difference in the structure; the product of secretion usually
appears granular, while the substance of the network is homo-
geneous.

As stated above, I consider this network as produced by the
transformation of the chondriosomes, not by a natural process,
as is well understood, but by an artificial one.   Of any inter-
vention on the part of the chondriosomes in the formation of the
secretion product, an intervention which in a number of cases
seems to me entirely plausible, nothing could be seen here.
Under what form does the product of secretion of the interstitial
cells make its appearance?   I have mentioned the presence of
granules that are preserved by a number of fixing reagents.
These granules are certainly secreted by the cell, but in my
opinion they are too scarce and inconstant to be considered as
the forerunners, or at least the only forerunners, of the product
of the cell.   One must consequently admit that the bulk of the
secretion makes its appearance in a diffuse or non-stainable form.
I would suggest, without, however, being able to substantiate that
opinion, that the product of secretion is represented by the sub-
stance which is responsible for the diffuse staining of the proto-
plasm, something like Mulon's osmiophile or siderophile substance.
But there is no indication in this case, as Mulon states it, that

this substance be formed by the coalescence of the chondriosomes, nor that the elimination be accompanied by a partial destruction of the cell.

While the existence of these strangely deformed chondriosomes is perhaps not very interesting in itself, it calls the attention of the cytologist to the possibility of a confusion between different cellular structures.   Regardless of whether or not my interpretation be correct, two things are evident: (1) That the network has nothing whatever to do with the apparatus of Golgi, as is shown after the application of Ramon y Cajal's method; (2) that it bears a remarkable resemblance to many of the structures described as the apparatus of Golgi.   There is one instance in the literature in which the chondriosomes, after application of a silver method, have been mistaken for the Golgi's apparatus (the cartilage cell, by Pensa, 1901),[1] and it is possible that a similar error is also the basis for Monti's interpretation of this structure in the adult nerve-cell.   According to that writer (1915), what was described in these cells as the apparatus of Golgi is identical with the chondriosomes.   I have discussed her paper extensively in another place (1919).   Having since discovered this network in the interstitial cells of the opossum I am inclined to believe that Monti has been misled by a similar appearance.

Another point which I feel should be touched upon in this connection is Holmgren's trophospongium theory.   In its latest form this theory claims the existence in a tissue of two types of cells—the parenchymal cells and the trophocytes.   The former are penetrated by processes of the trophocytes, processes which form the trophospongial net and become, under certain circumstances liquified, forming the "*Kanälchen des Trophospongiums*."   The rôle of the trophocytes is, as their name implies, a trophic one. While Holmgren himself has never published anything on the interstitial cells of the testicle, these are the only objects for which his theory has found whole-hearted support.   In one paper Bouin and Ancel (1905, 1) describe, in the first generation of interstitial cells in the testicle of horse fetuses from 22 to 42 cm. long, a structure which is "l'homologue du trophosponge de

[1] Pensa himself later on (1913) acknowledged his mistake.

Holmgren" (p. 395). This trophospongium belongs to the
diffuse type. It appears either as a canalicular network (p. 399),
or as a solid network (p. 404), and the authors suppose that it is
the "transformation substantielle de ce trophosponge qui donne
naissance au réseau des canalicules du suc, selon la conception
de Holmgren" (p. 404). In that paper Bouin and Ancel are
quite reserved as to the existence of connections between this
network and trophocytes, but they subscribe entirely to Holm-
gren's conception in a note published the same year (1905, 2),
evidently written subsequent to the other. According to their
description the young interstitial cells of the testicle of the horse
are penetrated by processes of cells of connective tissue acting as
trophocytes. These processes are later transformed into a system
of canals which open at the periphery of the interstitial cells.
In these later stages they still surround, but can no longer be
followed within the interstitial cells.

Whether or not Bouin and Ancel are still upholding their view
I do not know, but it should be pointed out that no mention of it,
or of the "canalicules du suc" is made in Bouin's description of
the interstitial cells of the testicle, in Prenant, Bouin and Mail-
lart's Traité d'Histologie. I would call attention also to the
fact that the only figure in the above mentioned papers (Fig. 3,
1905, 1) that shows anything like what the authors describe, in
reality rather contradicts that description. This figure repre-
sents a group of interstitial cells containing something that
resembles the "canalicules du suc"; but at the same time
there is no trace of trophocytes, cells of connective tissue, or any
connective tissue at all, between these cells, and consequently
no indication of a connection between such cells and the canalicu-
lar apparatus. This is the crucial point. Aimé (1907) also has
described in the interstitial cells of the ovary intracellular spaces
whose existence is beyond doubt, but he states definitely:
"Nous n'avons pas vu de relations entre ces canaux et les cellules
conjonctives entourant les cellules interstitielles" (p. 121, Fig.
11). The first point I would dispute is the existence of the tro-
phocytes, at least in the opossum. As stated above, in a heap
or row of interstitial cells the cells themselves are not separated
by connective tissue or cells of any kind. On the other hand, if

Bouin and Ancel met with intracellular accumulations of an unstainable product of secretion, or such deformed chondriosomes as I have described above, I can readily see, from my own observations, how they arrived at their interpretation.

Concerning variations in number, size or structure of the interstitial cells with the development of the testicle (the most recent investigations upon these points being those of Rasmussen, 1917), I have little to say.   The only thing which I noted was that, in the youngest testicles preserved (those in which the process of spermatogenesis had just begun) the interstitial cells were smaller than in the adult.

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EXPLANATION OF PLATES.

All figures were outlined with a Zeiss camera-lucida, at the level of the stage of the microscope. Lenses used: for Figs. 1–12, Zeiss apochr. immers. 2 mm. (ap. I, 40), in combination with ocular 12; for Fig. 13, Zeiss apochr. imm. 1 mm. 5 (ap. I, 30), and ocular 6. Artificial light (gas).

EXPLANATION OF PLATE I.

1. Interstitial cell with idiosome and 2 centrioles. Fixation and stain after Benda.

2. Interstitial cell with two nuclei, idiosome and 4 centrioles. Fixation and stain after Benda.

3. The apparatus of Golgi. Ramon y Cajal's method. Counterstain: Ehrlich's hematoxylin.

4. Another form of the apparatus of Golgi. Ramon y Cajal's method. Counterstain: methylgreen.

5. Interstitial cells with several crystals of the thin type. Fixation and stain after Benda.

6. A crystal of the thick type. Fixation and stain after Benda. The same cel shows part of the network made up of modified chondriosomes.
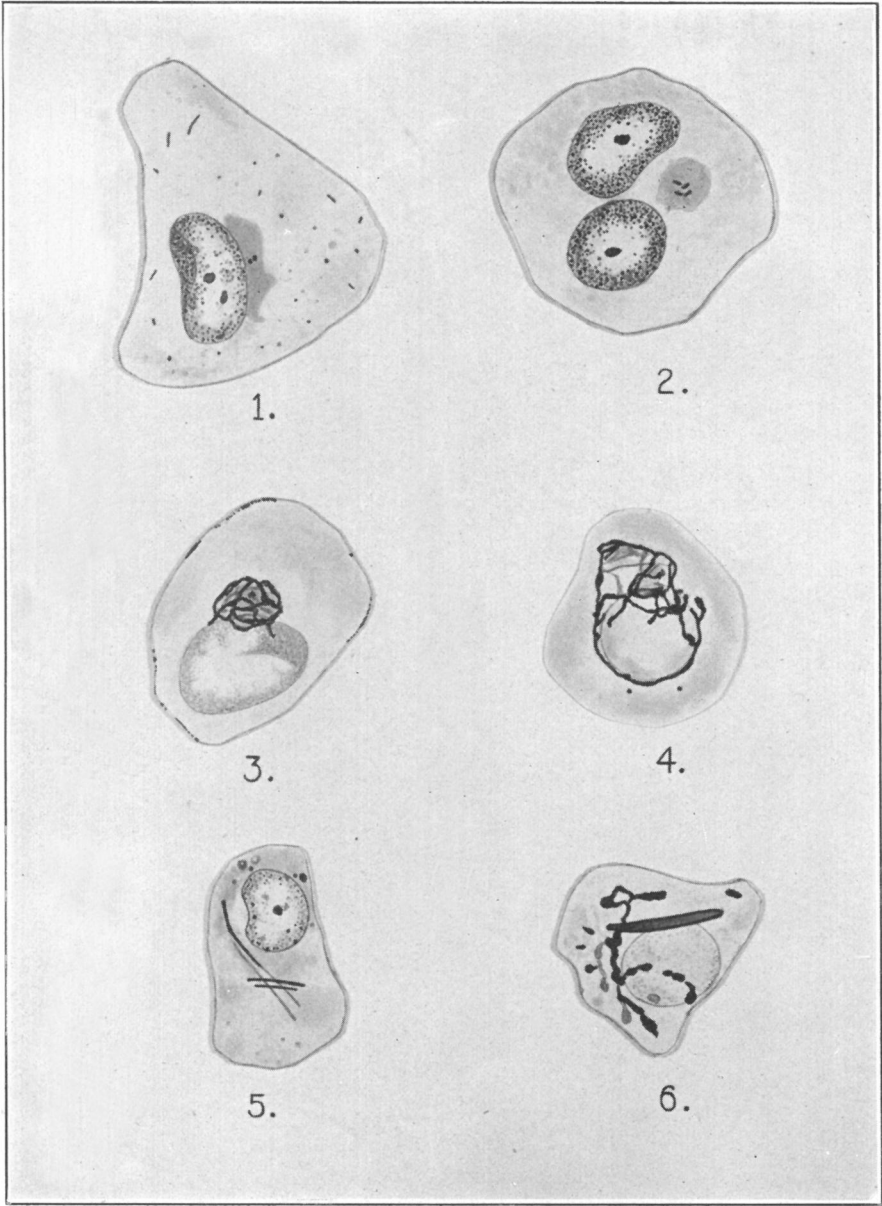
PLATE I.

7. Binucleated cell with normal chondriosomes.   Fixation and stain after Benda.

8. Cell with modified chondriosomes.   Same fixation and stain.

9, 10 and 11.   Different types of networks formed of modified chondriosomes. Same fixation and stain.

12. An interstitial cell with several exogenous processes.   Same fixation and stain.

13. A group of interstitial cells, with intracellular and intercellular accumulation of secretion-product.   The capillary, filled with the same product, extends, as the next section shows, up to the point marked X.   Same fixation and stain.

PLATE II.